

The Microbiota of a Mite Prey-predator System on Different Host Plants Are Characterized by Functional Redundancy and Dysbiosis

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Abstract

Microbiota have diverse roles in the life cycles of their hosts, affecting their growth, development, behavior, and reproduction. Changes in physiological conditions of the host can also impact the assemblage of host-associated microorganisms. However, little is known of the effects of host plant-prey-predatory mite interactions on mite microbiota. We compared the microbial communities of eggs and adult females of the two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) and of adult females of the predatory mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) on four different host plants (cotton, maize, pinto bean, and tomato) by metabarcoding sequencing of the V3–V4 region of the 16S ribosomal RNA gene (16S rRNA), using the Illumina MiSeq platform. Only the egg microbiota of *T. urticae* was affected by the host plant. The microbiota of the predatory mite *N. californicus* was very different from that of its prey, and the predator microbiota was unaffected by the different host plant-prey systems tested. Only the microbiota of the eggs of *T. urticae* carried *Serratia* as a high fidelity-biomarker. Biomarker bacteria were also detected in the microbiota of adult females of *T. urticae* and *N. californicus*, with different biomarkers in each host-plant species. The microbiota associated with eggs and adult females of *T. urticae* and adult females of *N. californicus* differed in their potential contributions to the host mite.

Introduction

Arthropods establish a diverse array of interactions with microorganisms, ranging from mutually beneficial (mutualism) to neutral (commensalism) or parasitic (parasitism) [1–3]. The microorganisms harbored within the body of arthropods include gut microbes and extracellular and intracellular symbionts. The host-microbe relationships can be obligatory or facultative depending on the level of codependence developed [4]. Obligate endosymbionts can provide hosts with essential nutrients, while facultative symbionts contribute to host resistance to natural enemies and alter the range of suitable food sources accessed by their hosts [5].

The intimate relationship between hosts and obligate endocytobionts has selected for more-cooperative symbionts and more-dependent hosts, leading to the reduction of bacterial genomes, as shown for the aphid bacterium *Buchnera aphidicola* (*Gammaproteobacteria, Erwiniaceae*). *Buchnera* has lost genes for non-essential amino acids, cell-surface components, gene regulation, and cell defense, while genes responsible for biosynthesis of amino acids that are essential for aphid hosts have been retained [5, 6]. Some facultative endosymbionts that manipulate host reproduction or sex determination [7] also affect several fitness traits of their hosts, including their behavior and immune response against natural enemies [8–10].

Free-living symbionts in the gut of arthropods are predicted to play a major role in host-plant adaptation, mainly because they inhabit the main food-processing environment and can respond relatively rapidly to induced changes or to the presence of phytotoxins in the host diet [11, 12]. The gut bacteria of insects have proven to aid hosts to degrade synthetic pesticides [13–15] and to circumvent host-plant protease

inhibitors [16], affecting the efficacy of these xenobiotics for insect control. Sharing the luminal space of the gut by a multispecies microbiota that is frequently exposed to new bacteria obtained from the environment also facilitates the acquisition of new genes through exchange of genetic material, which can potentially lead to the development of phenotypes with diverse metabolic profiles [12]. The maintenance of diverse facultative symbionts in host populations is thought to hinge mainly on the balance of selection between the costs and benefits of infection, and is subjected to different selection pressures, such as diet, environmental habitat conditions, host developmental stage, and social behavior [17–21].

The role of bacterial symbionts in the life of animal species drives the routes through which microorganisms are transmitted to next generations [5]. Obligate symbionts are usually transmitted vertically, often through transovarial transmission, while gut bacteria and facultative symbionts can be transmitted vertically or horizontally [4, 5]. In the case of horizontal transmission, the acquired microbes increase in density after each generation if there are fitness benefits to the host. Moreover, mechanisms that manipulate hosts or promote transmission fidelity might also promote associations and lead to speciation, affecting mate choice [22] or causing hybrid lethality [23].

The host microbiota can also be acquired *de novo* from species occupying different trophic levels. Predatory mites were reported to share 15% of their core bacterial taxa with those of the prey [24]. Nevertheless, some of the bacteria acquired from the prey can engage in pathogenic associations with predators, and affect fecundity, longevity, and response to plant volatiles of the new host. These negative effects on the fitness traits of predatory mites can lead to ineffective control of spider mites [25]. Praying mantids, on the other hand, harbor a gut microbiota distinct from that of the host, suggesting that their gut microbiota has a distinct phylogenetic profile from the prey microbiota [26]. Understanding the composition of the bacterial microbiota of predator and prey species targeted for control or used in mass-rearing systems allows detection of pathogenic bacteria in mass-rearing systems and provides opportunities for manipulation of the bacterial community to improve the health of predatory mites and their performance as biocontrol agents [24].

The two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important pest of a wide range of greenhouse and field crops around the world, feeding on over 1,100 plant species [27]. Because of the mite's high capacity to evolve resistance to synthetic organic acaricides, the use of biological control of *T. urticae* with predatory mites, such as *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) is a management strategy adopted worldwide [28]. However, little is known about the effects of host plant-prey-predatory mite interactions on the ecology of the microbiota of prey and predators on different host-plant systems. Here, we tested the hypothesis of vertical transmission of the microbiota from the prey to the predator on different host plants and analyzed the potential functional contributions of the associated microbiota. We used metabarcoding analysis of the V3-V4 region of the 16S rRNA gene to assess the composition of the microbiota of adult females and eggs of the two-spotted spider mite *T. urticae* and adult females of the predatory mite *N. californicus* reared on four different host plants.

Material And Methods

Colonies of phytophagous and predatory mites

A stock colony of *T. urticae* was maintained under controlled conditions (25±2°C, 60% R.H., 14 h photophase) on leaves of jack bean (*Canavalia ensiformis* L.). Adult females of *T. urticae* were transferred from the stock colony to four different host plants: *i*) cotton (*Gossypium hirsutum* L.); *ii*) maize (*Zea mays* L.); *iii*) pinto bean (*Phaseolus vulgaris* L.); and *iv*) tomato (*Solanum lycopersicum* L.). Each colony of *T. urticae* was divided into two sub-colonies: *i*) one to serve as a stock colony of the two-spotted mite on each host plant, and *ii*) another to serve as a trophic system in which the predatory mite *N. californicus* was included.

Assessing the microbiota of *T. urticae* and *N. californicus*

Sampling and DNA extraction

After two generations on different host plants and on each host plant-prey system, samples of eggs and adult females of *T. urticae* and adult females of *N. californicus* (respectively 30, 15, 15 /replicate/treatment) were collected from each colony and subjected to genomic DNA (gDNA) extraction, following Gilbert et al. [29]. The DNA extracted was quantified in a spectrophotometer (Biomate 3, Thermo Electron Corp., Madison, WI) and stored at -20°C until further use. Samples were subjected to RNase A (Thermo Scientific™) treatment to remove any residual RNA, following the manufacturer's recommendations.

Amplification, library construction, and metabarcoding sequencing

gDNA of eggs and adult females of *T. urticae* and adult females of *N. californicus* was subjected to PCR amplification of the V3–V4 region of the 16S rRNA in a thermocycler programmed at 94°C for 2 min (1 cycle); 95°C for 45 s, 55°C for 1 min, and 72°C for 1.5 min (32 cycles); and 68°C for 10 min (1 cycle), with a final hold at 4°C. PCRs were run with 10 ng of gDNA, enzyme buffer (1 ×), 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.625 U Taq polymerase (GoTaq® DNA Polymerase), and 0.2 μM of each 16S Illumina primer in a final volume of 25 μL. Amplification efficiency was determined using 1.5% agarose gel electrophoresis, following standard procedures [30]. A second amplification reaction was performed using the commercial product Nextera XT DNA Library Preparation Kit (Illumina) for addition of Illumina indexers and adapters. Amplicons were purified with AMPure XP beads, quantified using standard qPCR, and subjected to library preparation using equal amounts of amplicons. Samples were sequenced according to the paired-end protocol (2 × 250 bp) in the MiSeq Illumina platform at the Multiusers Center of Agricultural Biotechnology, Luiz de Queiroz College of Agriculture, University of São Paulo.

Microbiome analyses

Sequence analysis was performed using the *dada2* package [31] available for the R environment v.4.0.3 [32]. The reads obtained were subjected to quality filters for removal of residual primers and low-quality

sequences, following the parameters: reads smaller than 250 bp were discarded (*truncLen* = 250, 250), the maximum number of *N* allowed in the sequences was 0 (*maxN* = 0), and the maximum number of errors assumed by the analyses was 2 for forward and reverse reads [*maxEE* = c(2, 2)]. Reads were paired and possible chimeric sequences were removed. Then, sequences were aligned against the sequences available in the Silva138 database [33]. Afterward, sequences were classified as Amplicon Sequence Variants (ASVs), using the method described by Callahan et al. [34], considering 99% as the limit of similarity. Sequences with fewer than 10 counts and less than 10% prevalence in samples were discarded. Sequences with fewer than 100 counts were combined as “others” to better represent the data.

The ASVs were subjected to alpha-diversity analysis, using the Shannon diversity index [35]. Rarefaction curves were used to determine the quality of the sampling effort, using the depth limit of the smallest library (= 3,013 reads). Beta diversity was analyzed using the Jaccard index and weighted and unweighted Unifrac distance metrics [36–38]. The Jaccard and Unifrac distance matrices were used in hierarchical grouping, using the principal coordinates analysis (PCoA) for the beta-diversity analysis in the MicrobiomeAnalyst platform (<https://www.microbiomeanalyst.ca/>), using the nonparametric Kruskal-Wallis and Permanova tests, both at $p < 0.05$ [39, 40]. Hierarchical clustering was also used to produce heatmaps for data visualization, using Pearson as the distance measure and the mean as the clustering algorithm. Differential abundance analysis was performed using the *EdgeR* test [41] combined with the False Discovery Rate (FDR) method ($p < 0.05$). The Linear Discriminant Analysis Effect Size (LEfSe) method was performed using the nonparametric factorial Kruskal-Wallis rank sum test to detect features with significant differential abundance, followed by the Linear Discriminant Analysis (LDA) to estimate the effect size of each differentially abundant feature [42].

ASV sequences and abundances were processed using *Piphillin* (<https://piphillin.secondgenome.com/>) [43, 44] to predict the functional contribution of each microbiota and allow identification of biomarkers. Comparative analyses of the predicted functions for each microbiota obtained in the *Piphillin* output were also conducted in the MicrobiomeAnalyst platform [39, 40], using *EdgeR* [41] combined with the False Discovery Rate (FDR) method ($p < 0.05$).

Results

Data pre-processing

The metabarcoding sequencing of the 16S rDNA of the microbiota associated with eggs and adult females of *T. urticae* and with adult females of *N. californicus* yielded a total of 2,902,789 reads, with a mean of 80,633 reads per sample. After quality filtering, the total number of paired reads was 591,495 with a mean length of 454 bp. Rarefaction curves demonstrated that the sampling efforts were adequate to fully represent the richness of the communities analyzed, as no increase in ASV diversity was observed or unique ASVs detected after sampling 3,013 sequences (Fig. S1).

Taxonomic profiles

The host plants did not affect the relative abundance of the microbiota associated with eggs and adult females of *T. urticae* and adult females of *N. californicus* at the phylum level. In the microbiota of *T. urticae*, *Proteobacteria* comprised nearly 99% of all bacteria detected, with *Firmicutes* and *Bacteroidota* comprising less than 1% of ASVs. *Proteobacteria* was also the most prevalent (> 85% of all ASVs) in *N. californicus*, with a higher relative abundance of *Firmicutes*, mainly in samples from maize and tomato, and *Bacteroidota* in samples from cotton and pinto bean (Fig. S2).

Proteobacteria was represented only by *Alphaproteobacteria* and *Gammaproteobacteria*. *Alphaproteobacteria* comprised more than 90% of the total microbiota associated with adult females of *T. urticae*, except for the microbiota of adults from cotton, in which the abundance of *Gammaproteobacteria* increased to around 35% of the total ASVs detected (Fig. 1a). The microbiota of eggs of *T. urticae* was dominated by *Gammaproteobacteria*, which comprised more than 95% of the relative abundance of all classes of bacteria detected, regardless of the host plant (Fig. 1a). *Gammaproteobacteria* was also the most prevalent in adult females of *N. californicus*, but with a lower relative abundance than observed in eggs of *T. urticae*. *Alphaproteobacteria* predominated in the microbiota of *N. californicus* from maize, while *Gammaproteobacteria* predominated in the microbiota of predatory mites from cotton (Fig. 1a). *Firmicutes* was consistently recorded in samples of predatory mites from maize and tomato, but only sporadically represented in samples of predatory mites from cotton and pinto bean (Fig. 1a).

Serratia (*Gammaproteobacteria*, *Enterobacteriaceae*) dominated the microbiota of *T. urticae* eggs from all host plants (Fig. 1b). In contrast to eggs, the microbiota of adult females of *T. urticae* was dominated by the alphaproteobacterium *Rickettsia* (*Rickettsiaceae*), comprising more than 75% of the relative bacterial diversity (Fig. 1b). The prevalence of *Rickettsia* in the microbiota associated with *T. urticae* females was shared only with *Pseudomonas* (*Gammaproteobacteria*, *Pseudomonadaceae*) in cotton and with *Wolbachia* (*Alphaproteobacteria*, *Anaplasmataceae*) in tomato (Fig. 1b). The microbiota associated with adult females of *N. californicus* was more diversified, but *Serratia*, *Rickettsia*, and *Pseudomonas* were the most abundant bacteria, changing in relative abundance from host plant to host plant (Fig. 1b). Several other genera were detected in *N. californicus* females, including *Paenibacillus* (*Bacilli*, *Paenibacillaceae*), common in the microbiota of this mite from tomato (Fig. 1b).

Alpha and beta diversities

The alpha-diversity analysis indicated that host plants did not affect the microbial community diversity associated with eggs and adult females of *T. urticae* or with adults of *N. californicus*, except for the microbiota associated with *T. urticae* females on pinto bean (Shannon index values: $F = 6.05$; $p = 0.04$). The alpha-diversity of the microbiota associated with eggs of *T. urticae* on pinto bean was also lower than the diversity observed in adults of *N. californicus* (Shannon index values: $F = 4.36$; $p = 0.0471$) (Fig. 2). Other comparisons did not result in significant differences for alpha diversity (Table S1).

PCoA analysis using Jaccard indices indicated that the microbiota of *T. urticae* from maize, pinto bean and tomato were closely clustered, while the microbiota from cotton was more dispersed (Fig. 3a; 3b).

PCoA analysis indicated no differences in the beta diversity of the microbiota of *N. californicus* in the host plant-prey systems evaluated (Fig. 3c). PCoA analysis using the distances based on weighted Unifrac showed that the microbiota associated with adult females of *T. urticae* from cotton differed most from the others (Fig. 3d), while samples from eggs of *T. urticae* were grouped (Fig. 3e), and samples from *N. californicus* were dispersed (Fig. 3f). PCoA analyses based on the unweighted Unifrac index separated the microbiota of adult females of *T. urticae* from maize and tomato into one group, and those from cotton and pinto bean in another group (Fig. 3g). However, the microbiota of eggs of *T. urticae* (Fig. 3h) and the predator *N. californicus* (Fig. 3i) clustered in accordance with the host plants.

Clustering analysis

Differences in ASV counts between species (*T. urticae* and *N. californicus*) and stages of development of *T. urticae* (eggs and adult females) contributed more in the clustering analysis than the differences in ASV counts among the host plants (cotton, maize, pinto bean, and tomato) (Fig. 4a). The members of *Proteobacteria* were over-represented in the microbiota of *T. urticae* compared to the microbiota associated with the predatory mite *N. californicus*, with *Gammaproteobacteria* predominating in eggs, and *Alphaproteobacteria* in adult females of *T. urticae* (Fig. 4b). The most abundant ASVs of the microbiota of *N. californicus* belonged to *Bacilli* and *Bacteroidia*, which were among the least represented ASVs in eggs and adult females of *T. urticae*.

Serratia was the most abundant ASV in the microbiota of eggs of *T. urticae*, but showed low abundances in the microbiota of adult females of *T. urticae* and *N. californicus* (Fig. 4c). *Rickettsia* was the most abundant ASV in adult females of *T. urticae*, followed by *Wolbachia*, with the exception of the microbiota of *T. urticae* reared on cotton and maize, in which *Wolbachia* had low abundances. *Pseudomonas* also appeared in high numbers in the microbiota of adult females of *T. urticae*, but was abundant only in mites reared on cotton plants (Fig. 4c).

The abundance of ASVs belonging to the microbiota associated with the predatory mite varied according to the host plant. *Pseudomonas* and *Flavobacterium* were the most abundant ASVs in *N. californicus* from cotton and pinto bean, while *Enterococcus*, *Bacillus*, and *Paenibacillus* were the most abundant in maize, and *Bacillus* and *Paenibacillus* in predatory mites reared on prey from tomato plants (Fig. 4c). *Stenotrophomonas* was also an abundant ASV in the microbial community associated with females of *N. californicus* from cotton, pinto bean, and tomato (Fig. 4c).

The dendograms produced using the distances based on weighted Unifrac showed that all samples of *T. urticae* eggs clustered in a clade separated from adult females of *T. urticae* (Fig. 5a) and *N. californicus* (Fig. 5b), except for one replicate of *T. urticae* eggs from cotton. This replicate clustered with one replicate of *N. californicus* from cotton (Fig. 5a) and two replicates of *T. urticae* eggs from cotton and tomato (Figure 5b). Adult females of *T. urticae* clustered according to the host plants, except for cotton leaves, while eggs of *T. urticae* and adult females of *N. californicus* did not cluster according to the host plants (Fig. 5a, 5b).

Comparative analysis

Comparative analysis showed that the microbiota associated with eggs of *T. urticae* was affected by the rearing host plant, while we detected no evidence that the microbiota associated with the predatory mite *N. californicus* and adult females of *T. urticae* suffered any effect from the host plant. The effect of the host plant on the diversity and richness of the microbiota associated with eggs of *T. urticae* was observed in mites reared on cotton. One unassigned genus was more abundant in the microbiota of eggs from cotton than from the other host plants.

Comparative analysis from maize indicated 4.5 times more *Serratia* in the microbiota of *T. urticae* eggs than in the microbiota of *N. californicus* females, but the microbiota of *N. californicus* had 4.3–6.7 times more *Rickettsia*, *Methylophilus*, and *Pseudomonas* than the microbiota of *T. urticae* eggs (Fig. 6). Eggs of *T. urticae* from pinto bean had 10 times more *Serratia* and 4 times more *Wolbachia* than females of *N. californicus* (Fig. 6). The abundance of *Serratia* and *Wolbachia* in the microbiota of eggs of *T. urticae* from tomato was also higher than in the microbiota of *N. californicus*, with *Candidatus Hamiltonella* showing an opposite trend (Fig. 6).

The microbiota of eggs had higher abundance of *Serratia* than the microbiota of adult females of *T. urticae* from all host plants (Fig. 7). The microbiota of adult females of *T. urticae* had from 4.5 to 6.2 times more *Rickettsia* than the microbiota of eggs from cotton (Fig. 7) and maize (Fig. 7). *Wolbachia* and *Enterococcus* were also more abundant in the microbiota of adult females than in the microbiota of eggs of *T. urticae* from cotton (Fig. 7), while the opposite was observed for the abundance of the ASV that represented an unassigned genus (Fig. 7).

LDA Effect Size (LEfSe) analyses identified biomarkers in the microbiota associated with *T. urticae* and *N. californicus* reared on different host plants. *Serratia* was identified as a biomarker for the microbiota of eggs of *T. urticae* regardless of the host-plant association, while the microbiota of adult females of *T. urticae* was characterized by *Rickettsia* in females from cotton and maize, *Rickettsia* and *Wolbachia* from pinto bean, and *Wolbachia* from tomato (Fig. 8). Biomarkers for the microbiota of the predatory mite were identified only when feeding on pinto bean- (*Pseudomonas*) and tomato-prey systems (*Paenibacillus*) (Fig. 8).

Functional prediction analysis

The relative abundance of the pathways predicted from the microbiota associated with eggs and adult females of *T. urticae* and adult females of *N. californicus* was similar regardless of the host-plant association (Fig. 9), except for the lower relative abundance of the *amino-acid metabolism* (AAM) and *xenobiotic biodegradation and metabolism* (XBM) pathways. The relative abundance of the AAM and XBM pathways of the microbiota from *T. urticae* females from maize, pinto bean, and tomato was half that in the remaining samples (Fig. 9).

Comparisons of the potential functional contribution between the microbiota of eggs of two-spotted spider mite and that of *N. californicus* females from each host plant indicated significant differences in the potential contribution of each microbiota (Table S2). Differences in the functional contribution changed from plant to plant. In cotton, we did not detect differences in the potential functional contribution for the microbiota of the predatory mite *N. californicus* compared to that of eggs of *T. urticae*. However, most comparisons between the microbiota of the predator and prey eggs indicated a higher potential functional contribution of the predator microbiota than the microbiota associated with eggs of *T. urticae* (Fig. 10). The contribution of the microbiota of eggs of *T. urticae* exceeded that of the microbiota of the predatory mite only in pathways related to *carbohydrate digestion and absorption* in maize, pinto bean, and tomato; *glycosphingolipid biosynthesis*, *linoleic acid metabolism*, and *various types of N-glycan biosynthesis* in maize; *arabinogalactan biosynthesis* in pinto bean; and the *calcium signaling* and *prolactin signaling* pathways in maize and pinto bean (Fig. 10).

We also observed differences in the functional contribution of the microbiota between eggs and adult females of *T. urticae* from all host plants tested (Fig. 10, Table S3). In general, a higher potential functional contribution was observed for microbiota of eggs of *T. urticae* than for that of adult females, particularly on maize, pinto bean, and tomato (Fig. 10). The egg microbiota functional contribution was higher than that of the microbiota of females in all host plants for the *phosphotransferase system (PTS)*, *other glycan degradation*, *various types of N-glycan biosynthesis*, *arabinogalactan biosynthesis*, and *glycosphingolipid biosynthesis* (Fig. 10). But the opposite was observed on maize, pinto bean, and tomato plants for pathways involved in *thermogenesis*, *zeatin biosynthesis*, *protein processing in the endoplasmic reticulum*, the *p53 signaling pathway*, and *apoptosis* (Fig. 10).

Discussion

The microbiota of eggs and adult females of the two-spotted spider mite *T. urticae* and of adult females of *N. californicus* were very distinctive, and only the egg microbiota was affected by the host plant. The microbiota of the predatory mite *N. californicus* was very different from that of the prey, and the diversity and abundance of microbiota on this mite were unaffected by the different host plant-prey systems tested. Biomarker bacteria were detected for both *T. urticae* and *N. californicus*, but only the microbiota of the egg stage of *T. urticae* showed high fidelity with the biomarker bacterium *Serratia* in the different conditions tested. Biomarker bacteria were detected in the microbiota of adult females of *T. urticae*, but the biomarkers were affected by the host plant used, while the microbiota of *N. californicus* was moderately impacted by the host plant-prey system used, and different biomarkers were detected, but only in tomato- and pinto bean-prey systems. The microbiota associated with eggs and adult females of *T. urticae* and adult females of *N. californicus* differed in their potential contributions to the host mite. Nevertheless, changes induced by the host plant in the microbiota of *T. urticae* and *N. californicus* did not produce communities with changes in their potential functional contributions.

Members of *Serratia* colonize a variety of niches and can either establish successful mutualistic associations or act as opportunistic pathogens [45, 46]. *Serratia* were also reported associated with the

ovarioles of *Gargara genistae* (F.) (Hemiptera: Membracidae), which suggests that it is vertically transmitted, playing a role in host metabolic complementation [47]. However, the presence of *Serratia* on the phylloplane of plants has also been reported, showing that *Serratia* may colonize plant surfaces without damaging the plant [48]. The prevalence of *Serratia* in eggs of *T. urticae* in all host plants tested and their low abundance in *T. urticae* adult females suggests that the association between *Serratia* and *T. urticae* is accidental, occurring through contact of the egg with plant surfaces. The high prevalence of *Serratia* in eggs of *T. urticae* significantly affected the potential functional contribution provided by the associated microbiota. Since we do not believe that eggs of *T. urticae* could harbor such a high abundance of a single bacterial type in the ooplasm, we suggest that the functional contribution of the egg microbiota of *T. urticae* is likely limited. This is also supported by the lack of indication that *Serratia* are primary symbionts and transferred to eggs by vertical transmission, as the adult microbiota of *T. urticae* is dominated by the *Alphaproteobacteria Rickettsia* and *Wolbachia*. These alphaproteobacteria are reported within oocytes and in cells adjacent to the eggs in the ovaries of *T. urticae*, clearly demonstrating a transovarial route of transmission [49, 50]. Bacteria transmitted transovarially are often present in low densities in host eggs but increase in density as the host grows and develops, as is the case with *Wolbachia* [51, 52].

Members of *Rickettsia* are known for their potential pathogenicity to vertebrates [53] but can also benefit invertebrates by increasing host fitness and the proportion of females, enhancing the immune response to pathogens, and increasing the heat-stress tolerance of the host [54, 55]. Previous studies showed that a *Rickettsia* strain associated with *Ixodes scapularis* Say (Acari: Ixodidae) possessed all the genes required for the synthesis of folate (vitamin B9), which are not present in the host genome. Folate is the most essential component for cell growth, participating in the one-carbon metabolism pathway in nucleotide synthesis and in the methylation of DNA, RNA, proteins, and phospholipids [56]. Although *Rickettsia* is horizontally transferred to some hosts [57, 58], we did not observe any pathological symptoms in our rearing colonies that would suggest *Rickettsia* would be pathogenic to adult females of *T. urticae*. We believe that *Rickettsia* act as a true symbiont of *T. urticae* because it has been reported to infect the ovaries and eggs of *T. urticae*, with no evidence of a pathological association [49].

Wolbachia can manipulate host reproduction phenotypes in arthropods via cytoplasmic incompatibility, parthenogenesis, male killing, and male feminization [7, 59, 60], but the environment can also influence the frequencies of endosymbionts [18]. Host plants play an important role in shaping the bacterial community of herbivores, which includes facultative bacterial endosymbionts. In a population of *Tetranychus truncatus* Ehara (Acari: Tetranychidae) from maize, the infection rates of *Wolbachia* were lower than those from tomato and soybean, showing that endosymbionts associated with *T. truncatus* are affected by host plants [61]. Our results showed that the relative abundance of *Wolbachia* in *T. urticae* increased from eggs to adult females, and that it was affected by the host plant. Host plants can reduce the prevalence of *Wolbachia* and the fecundity and hatching rates of *T. urticae*, indicating that host-plant suitability affects the prevalence of *Wolbachia* [62]. The reduced genome of members of *Wolbachia* due to the loss of some essential biosynthetic pathways imposes a nutritional burden on their hosts. The competition for key nutritional resources with hosts was shown to negatively impact host performance

and affect the host–*Wolbachia* association in low-quality host-plant sources, leading to *Wolbachia* elimination, as observed for *T. urticae* fed on cotton and maize leaves [51, 62].

The predatory mite *N. californicus* harbors a wide diversity of bacteria, with *Bacillus*, *Flavobacterium*, *Methylophilus*, *Paenibacillus*, *Pseudomonas*, *Rickettsia*, *Serratia*, *Stenotrophomonas*, and *Wolbachia* the as main constituents of its microbiota. The presence of some of these bacteria in the microbiota of eggs of *T. urticae* (*Serratia*) and adult females of *T. urticae* (*Pseudomonas*, *Rickettsia*, and *Wolbachia*) suggests that *N. californicus* may acquire them by horizontal transmission. The existence of a shared microbiota among interacting species belonging to different trophic levels suggests that bacteria can undergo horizontal transfer from one species to another through feeding. Horizontal acquisition of bacteria can occur directly through the consumption of host parts harboring microbes or indirectly through shared food sources with other species. However, analyses of a few host–predator associations demonstrated the existence of additional factors that limit the host microbiota from establishing as the predator microbiota, as in some cases the predator microbiota reflects that of the host [63, 64], but not in other cases [26].

Among the major constituents of the mite microbiota, *Rickettsia*, *Serratia*, and *Wolbachia* are endocytobionts, while *Bacillus*, *Flavobacterium*, *Methylophilus*, *Paenibacillus*, *Pseudomonas*, and *Stenotrophomonas* are free-living bacteria, commonly reported as associates of plants or the gut of arthropods [65–69]. Several species of *Pseudomonas* have been shown to benefit plants by alleviating certain stress conditions, and to provide, for example, plant protection [70–72]. Members of *Pseudomonas* have been investigated as biopesticides for control of mites [73, 74] and insects [75, 76]. *Pseudomonas* associated with plants that orally infect and kill herbivorous arthropods carry surface glycan decorations to escape the host humoral immune defense [77], killing the hosts by producing molecules with potent insecticidal activity and lytic enzymes (chitinases, phospholipases) (Flury et al. 2017; Vacheron et al. 2019).

Bacillus associated with predatory mite species were shown to aid digestion in mites, but they also inhibited mite population growth, suggesting the establishment of an opportunistic mode of pathogenesis [24, 79]. *Flavobacterium* and *Stenotrophomonas* were considered opportunistic pathogenic bacteria when in association with spider mites [67].

The effects of the host plant or host plant-prey systems on the gut microbiota of *T. urticae* and *N. californicus* did not produce significant differences in the potential functional contribution of their associated microbiota from plant to plant. However, the microbiota associated with each species or stage of development of *T. urticae* had very different potentials to functionally contribute to their hosts. The potential functional contribution of the microbiota associated with *N. californicus*, even by sharing some members of the microbial community with the host, was very distinct. The microbiota of *N. californicus*, particularly the free-living bacteria, provide a significant contribution to host nutritional metabolism (*protein digestion and absorption*, *fat digestion and absorption*, *mineral absorption*, *cholesterol metabolism*, *carotenoid biosynthesis*) and defense against pathogenic bacteria and fungi by contributing

to the production of bioactive molecules (*penicillin and cephalosporin biosynthesis, staurosporine biosynthesis, and biosynthesis of various secondary metabolites*).

In conclusion, we demonstrated that the microbiota associated with eggs and adult females of *T. urticae* are quite different one from another, and that the microbiota of the predator *N. californicus* does not resemble the microbiota of the prey. We also demonstrated that the microbiota of *T. urticae* is little affected by the host plant, and the microbiota of *N. californicus* has little impact on the host plant-prey system used, both characterized by a discrete dysbiosis effect. Our data also indicated that *Rickettsia* is a primary symbiont of *T. urticae*, and that both *T. urticae* and *N. californicus* carry potentially pathogenic *Pseudomonas* species. We also demonstrated that the microbiota of *N. californicus* has a much higher potential to contribute secondary molecules that would protect the host against pathogenic bacteria and fungi than the microbiota associated with *T. urticae*. The association of *Wolbachia* with *T. urticae* and *N. californicus* was severely affected by the host plant. We also concluded that host plants can induce dysbiosis in the microbiota associated with *T. urticae* and *N. californicus*, but changes observed in the microbiota of these mite species from plant to plant did not produce changes in the functional contribution of the associated microbiota, demonstrating the occurrence of redundancy in the functional contributions of the observed dysbiotic microbiota.

Declarations

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Conflicts of interest/competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

BLM: conceptualization, methods, formal analysis, writing, original draft. GJM: experimental design, writing, revision. FLC: conceptualization, writing, revision and editing, project administration, funding acquisition, supervision.

Availability of data and material

Not applicable.

Ethical approval

The article does not contain any human and animal rights.

Consent to participate/publish

All authors agreed with their authorship, the final version of the manuscript and with its publication.

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Sequence data

The raw reads obtained by Illumina MiSeq sequencing were deposited in the Sequence Read Archive (SRA) at the National Center for Biotechnology Information under the accession number PRJNA784594.

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Figures

Figure 1

Relative abundance of the bacterial taxon hits at the (A) class and (B) genus taxonomic levels in the microbiota associated with eggs and adult females of *Tetranychus urticae*, and with adult females of the predatory mite *Neoseiulus californicus* reared on cotton, maize, pinto bean, and tomato as host plants

Figure 2

Shannon indexes of the bacterial communities associated with eggs and adult females of *Tetranychus urticae* and with adult females of the predatory mite *Neoseiulus californicus* reared on different host plants

Figure 3

Principal coordinates analysis (PCoA) from the distance matrix generated by Jaccard (A, B, C) and weighted (D, E, F) and unweighted (G, H, I) Unifrac indices for comparisons among adult females of *Tetranychus urticae* (B, D, G), eggs of *T. urticae* (B, E, H), and adult females of *Neoseiulus californicus* (C, F, I) from different host plants

Figure 4

Heatmaps clustering taxonomic abundance at ASV (A), class (B), and genus (C) levels in samples of eggs and adult females of *Tetranychus urticae* and of adult females of the predatory mite *Neoseiulus californicus* reared on different host plants

Figure 5

Dendrogram produced using the distances based on weighted Unifrac for microbiota associated with (A) eggs and adult females of *Tetranychus urticae* and (B) eggs of *T. urticae* and their predator *Neoseiulus californicus* reared on different host plants

Figure 6

Box plot representation for genera that showed significant differences in abundance between the microbiota of eggs of *Tetranychus urticae* and females of *Neoseiulus californicus* reared on maize, pinto bean, and tomato leaves

Figure 7

Box-plots representation for genera that showed significant differences in abundance between the microbiota of eggs and adult females of *Tetranychus urticae* reared on cotton, maize, pinto bean, and tomato leaves

Figure 8

LEfSe analysis of the bacterial biomarkers associated with eggs and adult females of *Tetranychus urticae* and with adult females of *Neoseiulus californicus*. Cladogram represents the hierarchical taxonomic structure from phylum to genus of the biomarkers identified

Figure 9

Relative abundances of the metabolic pathways represented in eggs and adult females of *Tetranychus urticae* and in adult females of *Neoseiulus californicus* from cotton, maize, pinto bean, and tomato host plants

Figure 10

Potential functional contributions of the microbiota associated with eggs and adult females of *Tetranychus urticae* and with the predatory mite *Neoseiulus californicus* in several pathways when mites are maintained on different host plants

Supplementary Files

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